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Group Art Unit: 1638 @ (703)- 308-0280
Title of the Invention: PROTEIN COMPLEMENTATION IN TRANSGENIC PLANTS

FIGURE 1A

Barnase coding sequence

```
                    met ala gln val ile asn thr phe asp gly val ala asp tyr leu gln thr tyr
          TCTAGACC  ATG GCA CAG GTT ATC AAC ACG TTT GAC GGG GTT GCG GAT TAT CTT CAG ACA TAT
3'gttcatgagatctgg   tac 5' (B1 primer)

                    his lys leu pro asp asn tyr ile thr lys ser glu ala gln ala leu gly trp
                    CAT AAG CTA CCT GAT AAT TAC ATT ACA AAA TCA GAA GCA CAA GCC CTC GGC TGG
                                                    (B4 primer)  3'  t gtt cgg gag ccg acc5'

                    val ala ser lys gly asn leu ala asp val ala pro gly lys ser ile gly gly
                    GTG GCA TCA AAA GGG AAC CTT GCA GAC GTC GCT CCG GGG AAA AGC ATC GGC GGA
                      5'gca tca aaa ggg aac c 3' (B2 primer)

                    asp ile phe ser asn arg glu gly lys leu pro gly lys ser gly arg thr trp
                    GAC ATC TTC TCA AAC AGG GAA GGC AAA CTC CCG GGC AAA AGC GGA CGA ACA TGG

                    arg glu ala asp ile asn tyr thr ser gly phe arg asn ser asp arg ile leu
                    CGT GAA GCG GAT ATT AAC TAT ACA TCA GGC TTC AGA AAT TCA GAC CGG ATT CTT

                    tyr ser ser asp trp leu ile tyr lys thr thr asp his tyr gln thr phe thr
                    TAC TCA AGC GAC TGG CTG ATT TAC AAA ACA ACG GAC CAT TAT CAG ACC TTT ACA

                    lys ile arg OCH
                    AAA ATC AGA taa
```

FIGURE 1B

Intergenic sequence

CGAAAAAAAACGGCTTCCTGCGGAGGCCGTTTTTTTCAGCTTTACATAAAGTGTGTAATAAATTTTTCTTCAAACTCTGATCGGTCAATTT
CACTTTCCGGATCCGGTCCAATCTGCAGCCGTCCGAGACAGGAGGACATCGTCCAGCTGAAACCGGGGCAGAATCCGGCCATTTCTGAAG
AGAAAAATGGTAAACTGATAGAATAAAATCATAAGAAAGGAGCCGCAC

FIGURE 1C

Barstar coding sequence

```
1   Met lys lys ala val ile asn gly glu gln ile arg ser ile ser asp leu his
2   ATG AAA AAA GCA GTC ATT AAC GGG GAA CAA ATC AGA AGT ATC AGC GAC CTC CAC
3

1   gln thr leu lys lys glu leu ala leu pro glu tyr tyr gly glu asn leu asp
2   CAG ACA TTG AAA AAG GAG CTT GCC CTT CCG GAA TAC TAC GGT GAA AAC CTG GAC
3

1   ala leu trp asp cys leu thr gly trp val glu tyr pro leu val leu glu trp
2   GCT TTA TGG GAT TGT CTG ACC GGA TGG GTG GAG TAC CCG CTC GTT TTG GAA.TGG
3

1   arg gln phe glu gln ser lys gln leu thr glu asn gly ala glu ser val leu
2   AGG CAG TTT GAA CAA AGC AAG CAG CTG ACT GAA AAT GGC GCC GAG AGT GTG CTT
3

1   gln val phe arg glu ala lys ala glu gly cys asp ile thr ile ile leu ser
2   CAG GTT TTC CGT GAA GCG AAA GCG GAA GGC TGC GAC ATC ACC ATC ATA CTT TCT
3

1   OCH
2   TAA TACGATCAATGGGAGATGAACAATATAGATCCCCCGGGCTGCAGGAATTC
3  5'taa tacgatcaatgggagatg 3' (B3 primer)
```

1: Translation of DNA sequences encoding Barnase (A) and Barstar (C), respectively
2: DNA sequence encoding either Barnase (A), Barstar (C) or the synthetic intergenic region (B) according to Paul et al. (1992)
3: Sequence of DNA primers that were used for IPCR to construct pepA* (B3/B4) and pepB* (B1/B2).

FIGURE 1D

Translational fusion of
ORF Peptide A**/ (Gly4 ser) 3 Linker peptide / GUS

```
         met ala gln val ile asn thr phe asp gly val ala asp tyr leu gln thr tyr his lys
tctagacc ATG GCA CAG GTT ATC AAC ACG TTT GAC GGG GTT GCG GAT TAT CTT CAG ACA TAT CAT AAG

         leu pro asp asn tyr ile thr lys ser glu ala gln ala leu gly trp met gly gly gly
         CTA CCT GAT AAT TAC ATT ACA AAA TCA GAA GCA CAA GCC CTC GGC TGG ATG GGC GGT GGC

         gly ser gly gly gly gly ser gly gly gly gly ser gly ile pro gly tyr gly gln ser
         GGT TCC GGT GGC GGT GGC AGC GGC GGC GGT GGT AGC GGg atc ccc ggg tac ggt cag tcc

         pro met
         ctt atg ... of GUS
```

Underlined: ORF of peptide A**

FIGURE 1E

Nucleotide Sequence of Translational fusion of
Ubiquitin genomic sequence and ORF Peptide A***

```
  tctagacc ATGCAGATCT TCGTGAAAAC CTTGACCGGC AAGACCATCA CTCTCGAGGT CGAGAGCAGC GACACCATCG
ACAATGTCAA GGCCAAGATC CAAGACAAAG AAGGTATCAT TCTTCCTCAC TCAATCTGGA TTCTTCTCTT TAGCTTTTTG
AAATTCAGAT CTCTTATCAT TTACTTGTTT CTCCTTTAAG GAATCCCTCC GGATCAGCAG AGATTGATCT TCGCCGGAAA
GCAGCTCGAA GATGGCCGTA CTTTGGCTGA CTACAACATC CAGAAAGGTA CGAAATCATC CGAATCCTTC TGTTGATCAT
TTCGATGATC TGATTGTATA AACTCTAATG GATTGTTATC ATTTGTAAAC AGAATCTACA CTTCATCTTG TGTTGAGGCT
TAGAGGTGGA GCACAGGTTA TCAACACGTT TGACGGGGTT GCGGATTATC TTCAGACATA TCATAAGCTA CCTGATAATT
ACATTACAAA ATCAGAAGCA CAAGCCCTCG GCTGGATGTA Gaggatcc
```

Underlined: Introns A and B within the ubiquitin sequence.
Bold: glycine codon 76 at the end of the ubiquitin ORF

FIGURE 1F

Nucleotide Sequence of Translational fusion of
Ubiquitin genomic sequence and ORF Peptide B***

```
  tctagacc ATGCAGATCT TCGTGAAAAC CTTGACCGGC AAGACCATCA CTCTCGAGGT CGAGAGCAGC GACACCATCG
ACAATGTCAA GGCCAAGATC CAAGACAAAG AAGGTATCAT TCTTCCTCAC TCAATCTGGA TTCTTCTCTT TAGCTTTTTG
AAATTCAGAT CTCTTATCAT TTACTTGTTT CTCCTTTAAG GAATCCCTCC GGATCAGCAG AGATTGATCT TCGCCGGAAA
GCAGCTCGAA GATGGCCGTA CTTTGGCTGA CTACAACATC CAGAAAGGTA CGAAATCATC CGAATCCTTC TGTTGATCAT
TTCGATGATC TGATTGTATA AACTCTAATG GATTGTTATC ATTTGTAAAC AGAATCTACA CTTCATCTTG TGTTGAGGCT
TAGAGGTGGA GCATCAAAAG GGAACCTTGC AGACGTCGCT CCGGGGAAAA GCATCGGCGG AGACATCTTC TCAAACAGGG
AAGGCAAACT CCCGGGCAAA AGCGGACGAA CATGGCGTGA AGCGGATATT AACTATACAT CAGGCTTCAG AAATTCAGAC
CGGATTCTTT ACTCAAGCGA CTGGCTGATT TACAAAACAA CGGACCATTA TCAGACCTTT ACAAAAATCA GATAA...
```

Underlined: Introns A and B within the ubiquitin sequence.
Bold: glycine codon 76 at the end of the ubiquitin ORF

FIGURE 1G

DNA sequence of T PCR primers (example 1)

B5	5'	CACAAGTACTCTAGACCATG 3'	(forward)
B6	5'	CATCCAGCCGAGGGCTTGT 3'	(reverse)
B7	5'	GGCGGTGGCGGTTCCG 3'	(forward)
B8	5'	CCACTAGTTCTAGAGTACTTGTG 3'	(reverse)
B9	5'	GCACAGGTTATCAACACG 3'	(forward)
B10	5'	GCGGATCCTCTACATCCAGCCGAGGGCTTGT 3'	(reverse)
B11	5'	GCATCAAAAGGGAACC 3'	(forward)
B12	5'	GGTCTAGAGTACTTGTG 3'	(reverse)
Ubq16F	5'	GCTCTAGACCATGCAGATCTTCGTGAAAAC 3'	(forward)
UbqlR	5'	CTGGATCCACCTCTAAGCCTCAACA 3'	(reverse)
Ubqla	5'	TATGGATCCCCCGGGCTGCAGGAA 3'	(forward)
Ubqlb	5'	TCCACCTCTAAGCCTCAACAC 3'	(reverse)

SCHEMATIC ILLUSTRATION OF pepA AND pepB* CONSTRUCTION BY INVERSE PCR (IPCR)*

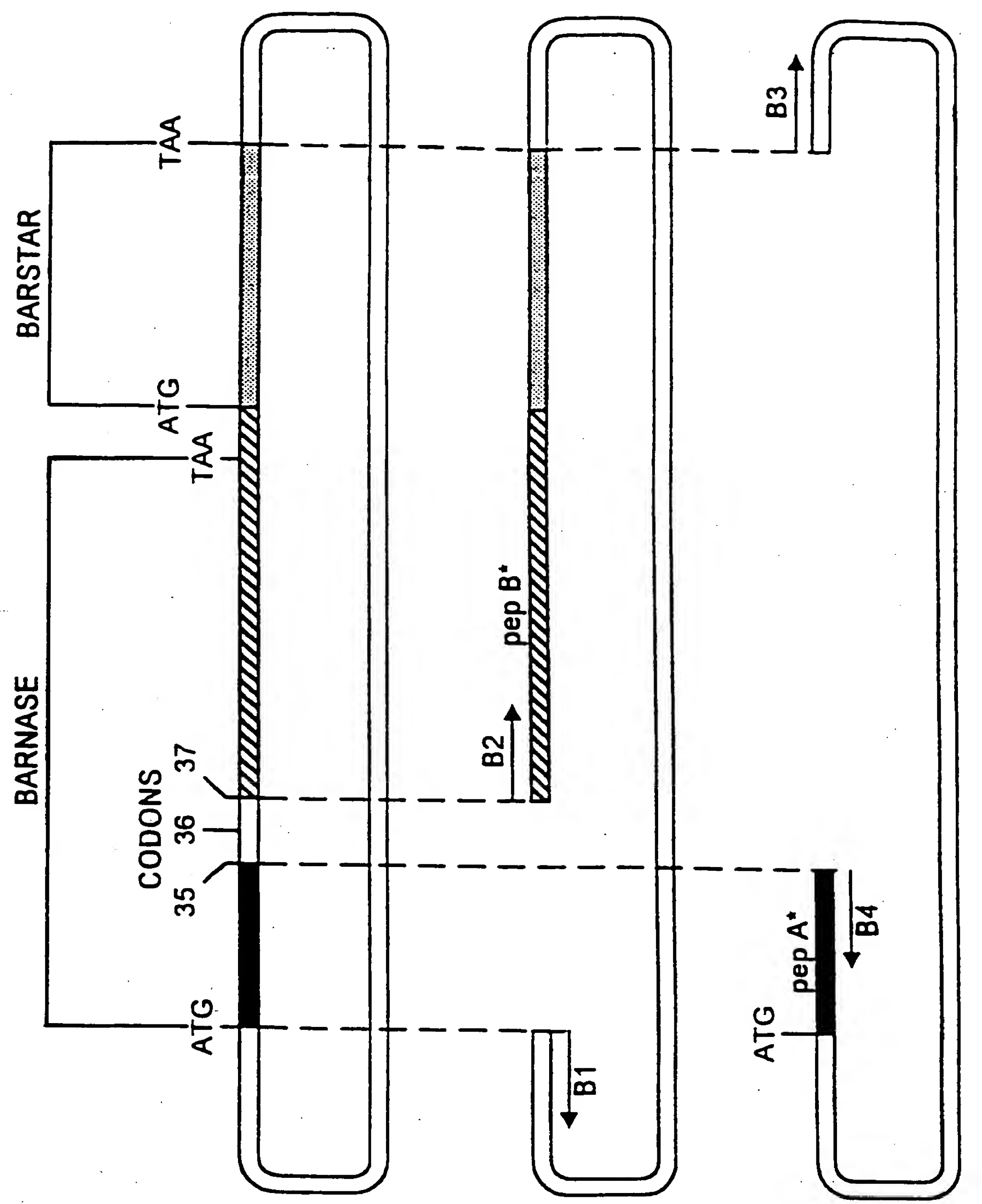

FIGURE 2

FIGURE 3A In Vitro Construction from Synthetic Obligonucleotides of S-peptide, S(+5)-protein and S -protein

```
1.  5'-gcggatccATGAAGGAGACCGCC-3OH
2.  5'-gcggatccATGAAGGAGACCGCCGCCGCCAAGTTCGAGCGCCAGCACATGGACAGC-3OH   5P-TAAAGATCTATG...
3.                                                 3OH-GTACCTGTCG__________ATTTCTAGATAC-5'

4.  5'-ccagatctATG----AGCTCCTCCAACTACTG-3OH
5.  ...AGCACCTCCGCCGCCAGCTCCTCCAACTACTGCAACCAGATGATGAAGTCT-3OH   5P-AGGAACCTGA...
6.                                             3OH-ACTACTTCAGA__________TCCTTGGACT-5'

7.  ...CCAAGGACAGGTGCAAGCCAGTCAACACCTTCGTCCACGAGAGCCTGGC-3OH   5P-CGATGTCCAG...
8.                                             3OH-CTCGGACCG__________GCTACAGGTC-5'

9.  ...GCCGTCTGCAGCCAGAAGAACGTGGCCTGCAAGAACGG-3OH   5P-TCAGACCAACT...
10.                                 3OH-CGTTCTTGCC__________AGTCTGGTTGA-5'

11. ...GCTACCAGTCCTACAGCACCATGTCCATCACCGACTGCCGCGAGACCGG-3OH   5P-CTCCAGCAAG...
12.                                            3OH-GCTCTGGCC__________GAGGTCGTTC-5'

13. ...TACCCTAACTGCGCCTACAAGACCACCCAGGCCAACAAGCACATC-3OH   5P-ATTGTTGCCTG...
14.                                       3OH-GTTCGTGTAG__________TAACAACGGAC-5'

15.                                  3OH-CTGCGGAGGCAGATTtcctagggc-5'
16. ...CGAGGGTAACCCTTACGTGCCTGTCCACTTCGACGCCTCCGTCTAAaggatcccg-3OH
```

FIGURE 3B

In Vitro Construction from Synthetic Obligonucleotides of the Sequence encoding the S-peptide and the (Gly4-Ser) 3 Linker

```
1.  5'-gcggatccATGAAGGAGACCGCC-3OH
2.  5'-gcggatccATGAAGGAGACCGCCGCCGCCAAGTTCGAGCGCCAGCACATGGACAGC-3OH   5P-GCCGGTGG...
3.                                                  3OH-GTACCTGTCG__________CCGCCACC-5'

4.  ...CGGTTCCGGTGGCGGTGGCAGCGGCGGCGGTGGTAGCaagatcttcggg-3OH
5.                                   3OH-CCATCGttctagaagccc-5'
```

FIGURE 4A

Protein and DNA Sequences of S-peptide and S-peptide with (Gly-4 Ser) 3 Linker

```
1   ------ --- AAA GAG ACA GCA GCC GCA AAG TTT GAG CGT CAG CAT ATG GAT AGT --- --- --- --- --- ---
2   ------ MET lys glu thr ala ala ala lys phe glu arg gln his met asp ser OCH gly gly gly gly ser
3   ggatcc atg aag GAG acc gcc GCC gcc AAG ttc GAG cgc CAG cac ATG gac agc taa --- --- --- --- ---
4   ggatcc atg aag GAG acc gcc GCC gcc AAG ttc GAG cgc CAG cac ATG gac agc --- ggc ggt ggc ggt tcc

1          --- --- --- --- --- --- --- --- --- --- ------
2          gly gly gly gly ser gly gly gly gly ser ------
3          --- --- --- --- --- --- --- --- --- --- -agatct
4          ggt ggc ggt ggc agc ggc ggc ggt ggt agc aagatct
```

Legend to Figure 4 A:

1: DNA sequence of the synthetic Bovine Rnase A gene (codon 1 to 15) according to N. Vasantha and David Filpula (1989)
2: Translation of synthetic DNA sequences encoding Bovine RNase A
3: DNA sequence of the S-peptide coding sequence referred to in this invention
4: DNA sequence encoding the S-peptide with (gly4 ser)3 linker peptide referred to in this invention

FIGURE 4B

Protein and DNA Sequences of S(+5)-protein and S-protein

```
1            --- AGC ACC AGT GCT GCC AGT TCT TCC AAC TAC TGT AAC CAG ATG ATG AAG TCT AGA AAC TTG ACC AAG
2            met ser thr ser ala ala ser ser ser asn tyr cys asn gln met met lys ser arg asn leu thr lys
3     agatct atg AGC ACC tcc gcc GCC agc tcc TCC AAC TAC tgc AAC CAG ATG ATG AAG TCT agg AAC ctg ACC AAG
4     agatct atg --- --- --- --- --- agc tcc TCC AAC TAC tgc AAC CAG ATG ATG AAG TCT agg AAC ctg ACC AAG

1                GAC AGA TGT AAG CCA GTT AAC ACA TTT GTC CAC GAG AGT TTG GCT GAT GTC CAA GCC GTC TGC AGT
2                asp arg cys lys pro val asn thr phe val his glu ser leu ala asp val gln ala val cys ser
3                GAC agg tgc AAG CCA gtc AAC acc ttc GTC CAC GAG agc ctg gcc GAT GTC cag GCC GTC TGC agc
4                GAC agg tgc AAG CCA gtc AAC acc tcc GTC CAC GAG agc ctg gcc GAT GTC cag GCC GTC TGC agc

1                CAG AAA AAC GTT GCA TGC AAG AAC GGT CAA ACG AAC TGT TAC CAG AGT TAC AGC ACC ATG TCC ATC
2                gln lys asn val ala cys lys asn gly gln thr asn cys tyr gln ser tyr ser thr met ser ile
3                CAG aag AAC gtg gcc TGC AAG AAC GGT cag acc AAC tgc TAC CAG tcc TAC agc ACC ATG TCC ATC
4                CAG aag AAC gtg gcc TGC AAG AAC GGT cag acc AAC tgc TAC CAG tcc TAC agc ACC ATG TCC ATC

1                ACT GAC TGT CGT GAG ACA GGC TCG AGC AAG TAT CCT AAT TGT GCT TAC AAG ACC ACA CAG GCG AAC
2                thr asp cys arg glu thr gly ser ser lys tyr pro asn cys ala tyr lys thr thr gln ala asn
3                acc GAC tgc cgc GAG acc GGC tcc AGC AAG tac CCT aac tgc gcc TAC AAG ACC acc CAG gcc AAC
4                acc GAC tgc cgc GAG acc GGC tcc AGC AAG tac CCT aac tgc gcc TAC AAG ACC ACA CAG gcc AAC

1                AAA CAC ATC ATT GTT GCT TGT GAA GGT AAC CCT TAC GTT CCT GTC CAC TTT GAC GCC AGT GTT TAA
2                lys his ile ile val ala cys glu gly asn pro tyr val pro val his phe asp ala ser val OCH
3                aag CAC ATC ATT GTT gcc tgc gag GGT AAC CCT TAC gtg CCT GTC CAC ttc GAC GCC tcc gtc TAA
4                aag CAC ATC ATT GTT gcc tgc gag GGT AAC CCT TAC gtg CCT GTC CAC ttc GAC GCC tcc gtc TAA

1                -------
2                -------
3                aggatcc
4                aggatcc
```

Legend to Figure 4B :

1: DNA sequence of the synthetic Rnase A gene (codons 16 to 124) according to Vasantha and Filpula (1989)
2: Translation of DNA sequences encoding the Bovine RNase A
3: DNA sequence of the synthetic S(+5)-protein coding sequence (aa16 to aa124)
4: DNA sequence of the synthetic S-protein coding sequence (aa21 to aa124)

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FIGURE 4C

i. PCR amplification product encoding impartial AOX3 targeting signal

```
XbaI / BglII
tctagatcttaac ATGAAGAATG TTTTAGTAAG GTCAGCTGCG CGAGCTCTGC TTGGCGGCGG
              TGGGCGGAGC TACTACCGCC AGCTCTCAAC GGCGGCGATC GTGGAACAGA
              GACACCAGCA CGGTGGCGGC GCGTTTGGAA GCTTCCA cttaagcggatcc
                                                        AflII / BamHI
```

ii. ORF encoding AOX3 targeting sequence (underlined) and S-peptide

```
ATGAAGAATG TTTTAGTAAG GTCAGCTGCG CGAGCTCTGC TTGGCGGCGG TGGGCGGAGC
TACTACCGCC AGCTCTCAAC GGCGGCGATC GTGGAACAGA GACACCAGCA CGGTGGCGGC
GCGTTTGGAA GCTTCCACTT AAGAAGGATG AAGGAGACCG CCGCCGCCAA GTTCGAGCGC
CAGCACATGG ACAGCTAA
```

iii. ORF encoding AOX3 targeting sequence (underlined) and S-peptide-(Gly4 Ser)3-GUS

```
ATGAAGAATG TTTTAGTAAG GTCAGCTGCG CGAGCTCTGC TTGGCGGCGG TGGGCGGAGC
TACTACCGCC AGCTCTCAAC GGCGGCGATC GTGGAACAGA GACACCAGCA CGGTGGCGGC
GCGTTTGGAA GCTTCCACTT AAGAAGGATG AAGGAGACCG CCGCCGCCAA GTTCGAGCGC
CAGCACATGG ACAGCGGCGG TGGCGGTTCC GGTGGCGGTG GCAGCGGCGG CGGTGGTAGC
GGGATCCCCG GGTACGGTCA GTCCCTTATG --> GUS
```

iv. ORF encoding AOX3 targeting sequence (underlined) and S-protein

```
ATGAAGAATG TTTTAGTAAG GTCAGCTGCG CGAGCTCTGC TTGGCGGCGG TGGGCGGAGC
TACTACCGCC AGCTCTCAAC GGCGGCGATC GTGGAACAGA GACACCAGCA CGGTGGCGGC
GCGTTTGGAA GCTTCCACTT AAGAAGGATG AGCTCCTCCA ACTACTGCAA CCAGATGATG
AAGTCTAGGA ACCTGACCAA GGACAGGTGC AAGCCAGTCA ACACCTCCGT CCACGAGAGC
CTGGCCGATG TCCAGGCCGT CTGCAGCCAG AAGAACGTGG CCTGCAAGAA CGGTCAGACC
AACTGCTACC AGTCCTACAG CACCATGTCC ATCACCGACT GCCGCGAGAC CGGCTCCAGC
AAGTACCCTA ACTGCGCCTA CAAGACCACA CAGGCCAACA AGCACATCAT TGTTGCCTGC
GAGGGTAACC CTTACGTGCC TGTCCACTTC GACGCCTCCG TCTAA
```

v. Translational fusion of Ubiquitin genomic sequence and ORF of S-protein

```
ATGCAGATCT TCGTGAAAAC CTTGACCGGC AAGACCATCA CTCTCGAGGT CGAGAGCAGC
GACACCATCG ACAATGTCAA GGCCAAGATC CAAGACAAAG AAGGTATCAT TCTTCCTCAC
TCAATCTGGA TTCTTCTCTT TAGCTTTTTG AAATTCAGAT CTCTTATCAT TTACTTGTTT
CTCCTTTAAG GAATCCCTCC GGATCAGCAG AGATTGATCT TCGCCGGAAA GCAGCTCGAA
GATGGCCGTA CTTTGGCTGA CTACAACATC CAGAAAGGTA CGAAATCATC CGAATCCTTC
TGTTGATCAT TTCGATGATC TGATTGTATA AACTCTAATG GATTGTTATC ATTTGTAAAC
AGAATCTACA CTTCATCTTG TGTTGAGGCT TAGAGGtGGa tcCagCTCCA ACTACTGCAA
CCAGATGATG AAGTCTAGGA ACCTGACCAA GGACAGGTGC AAGCCAGTCA ACACCTCCGT
CCACGAGAGC CTGGCCGATG TCCAGGCCGT CTGCAGCCAG AAGAACGTGG CCTGCAAGAA
CGGTCAGACC AACTGCTACC AGTCCTACAG CACCATGTCC ATCACCGACT GCCGCGAGAC
CGGCTCCAGC AAGTACCCTA ACTGCGCCTA CAAGACCACA CAGGCCAACA AGCACATCAT
TGTTGCCTGC GAGGGTAACC CTTACGTGCC TGTCCACTTC GACGCCTCCG TCTAA
```

Underlined: introns A and B within the ubiquitin encoding sequence
Bold: codon for Glycine[76], marking the C-terminus of the ubiquitin.
Small letters: PCR introduced conservative codon changes to generate a BamHI site and to modify the codon usage

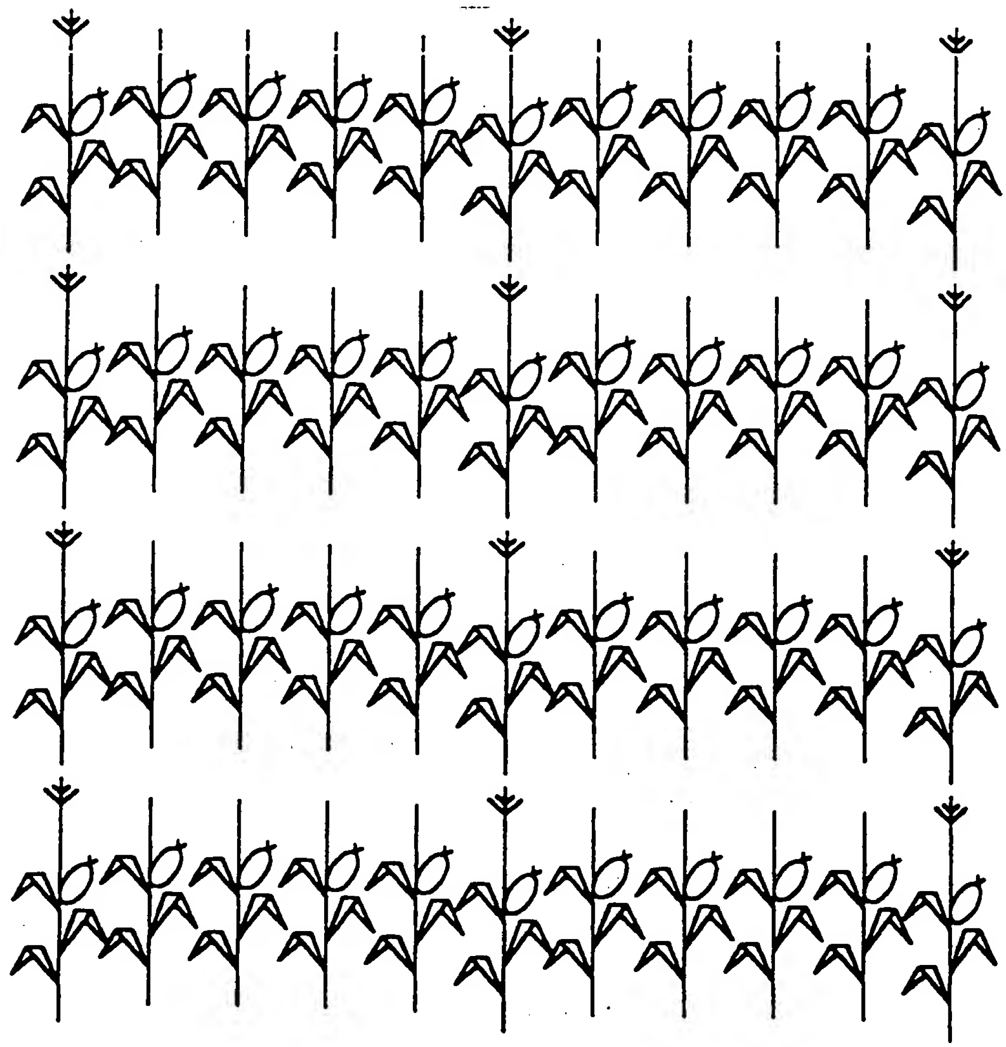
FIGURE 4D

Nucleotide sequence of PCR primers (example3)

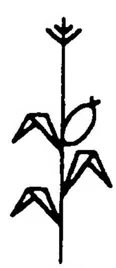
Sprot F	5' GGTGGATCCAGCTCCAACTACTGCAAC 3'
Sprot R	5' CGGGATCCTTAGACGGAGGCGTCG 3'
SprotMI1	5' GTCCTTAAGAAGGATGAGCTCCTCCAACTAC 3'
SprotMI2	5' CGGGATCCTTAGACGGAGGCGTCG 3'
SpepMI1	5' GTCCTTAAGAAGGATGAAGGAGACCGCCG 3'
SpepMI2	5' TCGGGATCCTTAGCTGTCCATGTGCTG 3'
SpepGMI2	5' TCGGGATCCTCATTGTTTGCCTCCCTG 3'
AOX3MI1	5' TGCTCTAGATCTTAACATGAAGAATGTTTTAG 3'
AOX3MI2	5' TCGGATCCGCTTAAGTGGAAGCTTCCAAAC 3'

FIGURE 5

FIGURE SHOWING A PRODUCTION SCHEME OF EMBRYO LESS MAIZE GRAINS: LINES A AND B ARE SHOWN IN ALTERNATIVE ROWS (FOR EXAMPLE ONE MALE AND FOUR FEMALES)



LEGEND
(REFER TO
DESCRIPTION
FOR DETAILS)



MALE PARENT A

FEMALE PARENT B